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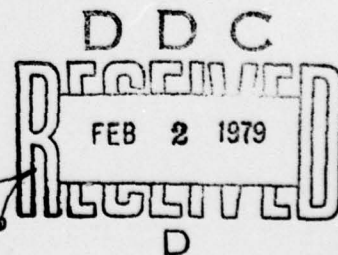
EFFECTS OF PROPELLANT HYDRAZINES ON RED BLOOD CELLS: METHEMOGLOBIN AND HEINZ BODY FORMATION

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DECEMBER 1978

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AEROSPACE MEDICAL RESEARCH LABORATORY
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FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AMRL-TR-78-127	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) EFFECTS OF PROPELLANT HYDRAZINES ON RED BLOOD CELLS: METHEMOGLOBIN AND HEINZ BODY FORMATION.		5. TYPE OF REPORT & PERIOD COVERED Technical Report
7. AUTHOR(s) MARILYN E. /GEORGE, WILLIAM E. /JOHNSON KENNETH C. /BACK Ph.D.		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102F 2312 V1-18
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) 9p.		12. REPORT DATE December 1978
		13. NUMBER OF PAGES 9
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) toxicology hydrazine monomethylhydrazine methemoglobin 1,1-dimethylhydrazine Heinz bodies		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Exposure to various hydrazine compounds causes methemoglobinemia and Heinz body production in human red blood cells. Three hydrazine compounds, monomethylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH) and hydrazine (Hz) are principal components of several Air Force propulsion systems and exposure to any of these compounds produces a hemolytic anemia. The anemia following MMH exposure is characterized by methemoglobin and Heinz body formation. This study was designed to determine if UDMH and Hz exposure also produce these effects and to		

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compare the effective dose levels. MMH is the most effective agent, producing these effects at concentrations eight to ten times lower than Hz. UDMH did not cause methemoglobinemia or Heinz bodies at the concentrations used indicating the probability of a different hemolytic mechanism. *K*

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PREFACE

This report represents research performed by the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from April 1978 to June 1978. The research was performed in support of Task 2312V1, "Toxic Substances in Air Force Environment," Work Unit 2312V118, "Effect of Air Force Propellants and Chemicals on Metabolic Mechanisms."

EFFECTS OF PROPELLANT HYDRAZINES ON RED BLOOD CELLS: METHEMOGLOBIN AND HEINZ BODY FORMATION

INTRODUCTION

Three hydrazine compounds, monomethylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH), and hydrazine (Hz) are principal components of several Air Force rocket propulsion systems and are known to be extremely toxic. Exposure to the individual hydrazines can cause a variety of toxic effects and pathological changes (Back et al, 1978) depending on the concentration of the compounds and the animal species exposed. Many physiological and biochemical parameters have been used to monitor exposures to these compounds but only the hematological system consistently shows evidence of deleterious effects from exposure to any of the three. All of these compounds cause an accelerated destruction of red blood cells although MMH is the most effective agent of the three in producing anemia (Clark et al, 1968). MMH causes a dose related hemolytic anemia characterized by a decrease in hemoglobin, hematocrit and red cell count, an increase in reticulocytes and the formation of Heinz bodies and methemoglobin following either intermittent or continuous exposure to low concentrations (MacEwen and Haun 1971). There is also a species difference in the red cell susceptibility to MMH. The hemolytic effect is most pronounced in the dog followed in decreasing order of severity by man, rat and monkey (Clark and DeLaGarza, 1967).

Previous investigations have shown that exposure of human red cells to MMH causes methemoglobinemia, Heinz body formation and changes in the cells' rheological properties which result in an increased rate of cell destruction. Electron microscopic examination of MMH exposed red cells suggested denatured hemoglobin exists in two forms: in a concentrated form as typical Heinz bodies visible by light microscopy and as a dispersed filamentous network seen by electron microscopy. Both forms contribute to an increased rigidity of the cells leading to sequestration and destruction in the spleen (Weinsten et al, 1975).

The study reported here was designed to determine whether UDMH and Hz as well as MMH produce methemoglobinemia and Heinz bodies which would suggest a similar mechanism of hemolytic action for the three compounds and to compare the levels of methemoglobin and Heinz bodies produced by equimolar amounts of UDMH, MMH and Hz.

MATERIALS AND METHODS

Whole blood was drawn from human subjects using heparin as an anticoagulant. The blood was centrifuged and the plasma and buffy coat discarded. The red cells were washed three times with isotonic phosphate buffered saline, pH 7.4, containing 0.011 M glucose and suspended to a hematocrit of 50%. The suspension contained less than 100 white cells/cu mm and the hemoglobin content was approximately 14.0g/100 ml. The molar concentrations of heme in all red cell suspensions were calculated and incubation mixtures of cells and the different hydrazines were made by the addition of sufficient MMH, UDMH or Hz to give the desired molar ratio of heme to toxicant; this ratio ranged from 1:1 to 64:1. The mixtures were incubated at 37°C and samples for methemoglobin and Heinz body determinations were taken at one, two and four hour intervals. In addition, the cell mixtures containing UDMH were

examined for Heinz bodies at six and twenty-four hours. Control blood samples were prepared the same way, incubated with isotonic buffered saline, and the determinations run concurrently. Heinz body formation was measured by supravital staining of the cells with 1% methyl violet in 0.9% saline and at least four preparations were examined at each time interval. Methemoglobin was determined by a modification of the Evelyn and Malloy method (Hainline, 1965).

RESULTS

The molar ratios of the heme to the different hydrazine compounds in the incubation mixtures were adjusted to 1:1, 2:1, 4:1, 8:1, 16:1, 32:1 and 64:1 and samples taken at one, two and four hours for methemoglobin determinations. The results are shown in Table 1. After a one hour exposure to MMH, methemoglobin was found in all samples with the amount decreasing at higher dilutions. At two and four hours methemoglobin was not found in samples at a ratio above the 16 μ M heme: 1 μ M MMH. Hydrazine also produced methemoglobin in red cells but only at comparatively high molar concentrations of hydrazine and the % methemoglobin was substantially less than produced by MMH. Methemoglobin was not found in samples with a dilution higher than 4 μ M heme: 1 μ M Hz at any time period. UDMH caused no methemoglobinemia at the concentrations tested. Control samples did not contain measurable methemoglobinemia at any sampling period.

TABLE 1

METHEMOGLOBIN

% of total hemoglobin

Molar Ratios heme:toxicant	1 Hour			2 Hour			4 Hour		
	MMH	Hz	UDMH	MMH	Hz	UDMH	MMH	Hz	UDMH
1:1	x	6.8	0	x	8.3	0	x	41.9	0
2:1	20.4	2.4	0	23.0	1.6	0	33.3	20.4	0
4:1	12.1	0.8	0	20.1	3.3	0	31.6	1.5	0
8:1	7.9	0	0	13.3	0	0	0	0	0
16:1	4.3	0	0	0	0	0	9.6	0	0
32:1	6.0	0	0	0	0	0	0	0	0
64:1	3.9	0	0	0	0	0	0	0	0

x - At this concentration denaturation and precipitation interfered with the measurement.

Cells from the same incubation mixtures were examined for the presence of Heinz bodies. Heinz bodies were found in cells from all samples exposed to MMH at ratios of heme to MMH of 16:1, 8:1, 4:1 and 2:1 after one, two and four hour exposures and in the sample with a ratio of 32:1 after four hours. The number of cells affected, the number of Heinz bodies per cell and the size of the Heinz bodies were directly related to concentration of MMH and length of exposure. At a 2:1 ratio of heme to MMH, 100% of cells contained multiple large Heinz bodies at one, two and four hour exposures; at 4:1 about 75% of

the cells contained two to five Heinz bodies at one hour but all cells were affected after two and four hours; at 8:1 about 50% of the cells contained at least one Heinz body after one hour with a progressive increase in number of cells affected and number per cell of Heinz bodies until 100% of the cells were affected at four hours; at 16:1 an occasional Heinz body was seen at one hour, 5-25% of cells contained one or two Heinz bodies at two hours and 25-50% of the cells had one or more Heinz bodies at four hours; at 32:1 the cells showed no Heinz bodies until four hours when 5-25% of the cells were affected. The red cells incubated with Hz showed a similar pattern but higher concentrations of Hz were required to produce Heinz bodies; at the ratio of 1:1 100% of the cells contained multiple Heinz bodies after two and four hours incubation. At a ratio of 2:1, only 5-25% of the cells contained one or more Heinz bodies after two hours, and about 75% of the cells were affected at four hours. At a ratio of 4:1, an occasional cell containing a Heinz body was seen at two hours and 5-25% of the cells were affected after four hours. There were no Heinz bodies seen in cells incubated with lower concentrations of Hz. The cells incubated with UDMH did not contain Heinz bodies at any of the concentrations examined. These cells were also examined at six and twenty-four hours exposure and were negative for Heinz body formation. Control cells exhibited a normal appearance with no Heinz body formation. These results are roughly quantitated in Table 2.

TABLE 2

HEINZ BODY FORMATION

Molar Ratios heme:toxicant	1 Hr			2 Hr			4 Hr		
	MMH	Hz	UDMH	MMH	Hz	UDMH	MMH	Hz	UDMH
1:1	x	+	0	x	4+	0	x	4+	0
2:1	4+	1+	0	4+	1+	0	4+	3+	0
4:1	3+	0	0	4+	+	0	4+	1+	0
8:1	2+	0	0	3+	0	0	4+	0	0
16:1	+	0	0	1+	0	0	2+	0	0
32:1	0	0	0	0	0	0	1+	0	0
64:1	0	0	0	0	0	0	0	0	0

- 4+ - 100% cells contain multiple Heinz bodies
- 3+ - 50-100% cells contain two to five Heinz bodies
- 2+ - 25-50% cells contain one or more Heinz bodies
- 1+ - 5-25% cells contain one or more Heinz bodies
- +
- occasional cell with one Heinz body
- x - at this concentration denaturation and precipitation interfered with the measurement.

DISCUSSION

The most consistent finding following either acute or intermittent exposure to MMH at either high or relatively low exposure concentrations is a hemolytic anemia characterized by a decrease in hemoglobin, red blood cell count, and hematocrit, reticulocytosis, methemoglobinemia and Heinz body formation. This anemia is observed when there are no other pathological changes or effects seen which indicates the hematopoietic system is one of the most sensitive to MMH and is

certainly the most easily measured. Two of the diagnostic procedures recommended following MMH exposure are measurement of methemoglobin and examination of red cells for Heinz bodies. Exposure to UDMH or Hz also produces anemia although these have not been as well defined as that following MMH. This study was done to compare the anemias produced by UDMH, MMH, and Hz and to determine if these anemias have similar analytical characteristics, i.e. methemoglobin and Heinz body formation, which can be recommended as diagnostic procedures following accidental exposures.

Our results indicate that UDMH exerts its hemolytic action through a different mechanism of action since no Heinz bodies or methemoglobin were observed. It is possible that UDMH is metabolized in vivo to a form which can produce these effects but we have not observed it in other studies in this laboratory. Conversely, Hz does produce Heinz bodies and methemoglobin but only at high concentrations, compared to MMH, making these determinations of somewhat doubtful practical value for Hz exposure. Results from previous studies indicated Heinz bodies and methemoglobinemia can be detected following exposure to 0.2 ppm MMH over a six month period or following an acute exposure to 90 ppm MMH for ten minutes. Since it requires approximately ten times higher concentration of Hz to produce the same effect, these tests are not as sensitive an indication of Hz exposure as of MMH exposure and negative results would have to be interpreted with caution.

REFERENCES

1. Back, K.C., V.L. Carter, Jr., and A.A. Thomas, "Occupational Hazards of Missile Operations with Special Regard to the Hydrazine Propellants," Aviat. Space Environ. Med. 49(4), 591-598, 1978.
2. Clark, D.A., J.D. Bairrington, H.L. Bitter, F.L. Coe, M.A. Medina, J.H. Merritt and W.N. Scott, "Pharmacology and Toxicology of Propellant Hydrazines," Review 11-68, School of Aerospace Medicine, Brooks Air Force Base, Texas.
3. Clark, D.A. and M. DeLaGarza, "Species Difference in Methemoglobin Levels Produced by Administration of Monomethylhydrazine," Proc. Soc. Exp. Biol. Med. 125:912, 1967.
4. Hainline, A., Jr., Standard Methods of Clinical Chemistry, Vol 5, Samuel Meites, Ed., Academic Press, N.Y. 1965.
5. MacEwen, J.D. and C.C. Haun, "Chronic Exposure Studies with Monomethylhydrazine," Proceedings of the 2nd Annual Conference on Environmental Toxicology, AMRL-TR-71-120, 255-270 Wright Patterson Air Force Base, Ohio, 1971.
6. Weinstein, R.S., M.E. George, and R.H. Steingart, "Contribution of Heinz Bodies to Alterations in Red Cell Deformability," Toxicol. Appl. Pharmacol. 32:545-558, 1975.